

## 論 文 要 旨

T-box Transcription Factor BRACHYURY and SOX2 Increase Self-Renewal and Invasive Phenotype in Adenoid Cystic Carcinoma: Implication for a New Therapeutic Principle

〔 T-box 転写因子 BRACHYURY と転写因子 SOX2 は腺様嚢胞癌の自己再生能と浸潤能を亢進する —新規治療法創出の可能性— 〕

中村 康大

### 【序論及び目的】

The high frequencies of recurrence and distant metastasis of adenoid cystic carcinoma (ACC) emphasize the need to better understand the biological factors associated with these outcomes. Recent studies suggest that epithelial–mesenchymal transition (EMT) correlates with cancer metastasis. In addition, there is growing evidence of the association of EMT with cancer stem cells (CSCs). Recently, we showed that the T-box transcription factor *BRACHYURY* could be a strong regulator of EMT. Further, we previously established the metastatic ACCS-M GFP cell line and reported that *SOX2* knockdown partially inhibits EMT phenotypes of ACCS-M GFP cells. Thus, in this study, we further tested whether *BRACHYURY* and *SOX2* is a regulator of cancer stemness by means of forced expression and silencing of these genes in adenoid cystic carcinoma (ACC) cell lines.

### 【材料及び方法】

*BRACHYURY*, *SOX2*, or both were transfected into ACC cell lines. Short hairpin RNA (shRNA) silencing of these genes was also performed. We analysed these cell lines with respect to self-renewal phenotypes using a sphere-formation assay, and we assessed the expression levels of EMT markers and stem cell markers using real-time reverse transcription-polymerase chain reaction (RT-PCR). Cell migration and invasiveness in vitro were evaluated using a wound healing assay and a tumor cell dissemination assay, respectively. Characteristics of CSCs were also analyzed by sphere-forming ability and *in vivo* tumorigenicity.

### 【結果】

Forced expression of *BRACHYURY* or *SOX2* slightly increased expression of EMT and stem cell markers and the self-renewal phenotype. The expression levels, however, were much lower compared to those of cancer stem cell-like cells. Forced co-expression of *BRACHYURY* and *SOX2* strongly upregulated EMT and stem cell markers and the self-renewal phenotype. Cell migration and invasiveness *in vitro* were also remarkably enhanced. These synergistic effects increased expression levels of *FIBRONECTIN*, *SNAIL*, *SLUG*, *ZEB1*, and *TGF- $\beta$ 2*. In particular, the effects on *FIBRONECTIN* and *TGF- $\beta$ 2* were significant. *Brachyury* knockdown significantly inhibited cell migration and invasion, and decreased tumorigenicity in ACC cells.

### 【結論及び考察】

We found that *BRACHYURY* and *SOX2* synergistically promote cancer stemness in ACC cells. This finding points to the importance of gene or protein networks associated with *BRACHYURY* and *SOX2* in the development and maintenance of the CSC phenotype. This study demonstrates that *Brachyury* knockdown reduces invasiveness of CSCs *in vivo*, suggesting that *Brachyury* silencing may be a useful therapeutic tool for salivary gland tumors including adenoid cystic carcinoma.